

Effect of biofertilizer, humic acid, irrigation with saline water and their interactions on some chemical and biological properties of the soil

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Abstract:

A field experiment was conducted in Najaf province at the University of Kufa - Agricultural Research Station for autumn season of 2023 AD in a loamy sand soil, classified according to the modern American classification as below the level of the supergroups Typic Torrifluvent according to the international classification. To study the effect of biofertilizer and humic acid on the growth and yield of corn under salt stress conditions. The experiment included three main factors, the first factor is to isolate a group of *Klebsiella oxytoca* bacteria that are tolerant to salt from the rhizosphere of the Tarfa plant, which is characterized by its high tolerance to salinity and is at two levels (without adding, adding) which represents (B1, B0). The second factor is to add humic fertilizer to the soil at levels (0, 4 and 8) kg. ha⁻¹ which is symbolized by (H2, H1, H0). The third factor is to irrigate the plants with water with salinity levels (1.5, 3 and 6) ds.m⁻¹ which represents (S2, S1, S0). The experiment was implemented according to the Randomized Complete Block Design (RCBD) with three replicates and the results of the experiment were statistically analyzed according to the analysis of variance (ANOVA) method and the significant differences between the averages of the treatments were calculated with the least significant difference at the level of 0.05 using the Genstat program in the statistical analysis. The results were as follows: The treatment of adding biofertilizer B1 significantly outperformed the other treatments and recorded the highest rate of available nitrogen, which reached 20.11 mg. kg⁻¹ soil, available phosphorus 26.40 mg P kg⁻¹ soil, available potassium 182.22 mg K kg⁻¹ soil, organic matter in the soil 11.06 g. kg⁻¹ soil, total number of bacteria in the rhizosphere soil 6.67 g⁻¹ soil. The results also showed that adding humic acid had a significant effect, as the H2 treatment with an addition level of 8 kg.ha⁻¹ was significantly superior in the characteristics of available nitrogen reaching 21.65 mg. kg⁻¹ soil, available phosphorus 32.41 mg P kg⁻¹ soil, available potassium 206.67 mg K kg⁻¹ soil, organic matter in the soil 12.18 g. kg⁻¹ soil, and the total number of bacteria in the rhizosphere soil 7.50 g⁻¹ soil. The results of irrigation with water with an electrical conductivity of 6 ds.m⁻¹ showed a decrease in the concentration of available nitrogen in the soil reaching 8.51 mg N kg⁻¹ soil, available phosphorus 20.42 mg P kg⁻¹ soil, available potassium 118.71 mg K kg⁻¹ soil, organic matter in the soil 8.47 g. kg⁻¹ soil, total number of bacteria in the rhizosphere soil 8.29 g⁻¹ soil ,

Introduction

Increasing agricultural production has become an urgent requirement for the growing population, however, the scarcity of fresh water is a constraint on irrigation worldwide, while saline water is abundant in the world and is an important resource for maintaining

agricultural irrigation. Saline irrigation has been widely used for plants such as wheat and sunflowers that tolerate moderate salinity [1] The problem of water scarcity is one of the most prominent challenges facing agriculture in many regions around the world, especially in dry and semi-dry areas, so agriculture using

saline water has become an option to meet this challenge, despite the challenges posed by this solution, as salinity leads to many problems for plants such as difficulty in absorbing water and nutrients, accumulation of harmful salts inside plant cells, reduced growth, and increased plant sensitivity to diseases. In this context, the importance of using sustainable agricultural techniques appears, such as adding biofertilizers that live in saline environments and are close to the roots of saline plants, as well as adding organic matter to improve soil properties and increase plant resistance to environmental stresses [2]

Biofertilizers are biological products that contain beneficial microorganisms such as bacteria and fungi that promote plant growth and improve soil health. These organisms play a vital role in improving the ability of plants to tolerate environmental stresses, including salinity, through several mechanisms, such as producing compounds that help mitigate salt toxicity. Bacteria in biofertilizers help plants better absorb nutrients, especially under salinity conditions that limit the ability of roots to absorb. Some bacteria also produce growth hormones such as auxin and gibberellins that stimulate the growth of roots and root hairs, increasing the absorption area and helping the plant obtain water and nutrients. Bacteria help improve soil structure by increasing organic matter and forming soil aggregates, which improves soil aeration and drainage [3] Humic acid is a complex organic compound found in soil and humus. Adding it to soil under saline conditions is an effective strategy to improve plant growth and increase yield and crop quality. This is due to its ability to improve soil properties by increasing the soil's ability to retain water and nutrients and increasing plant resistance to water and salt stress, protecting cell membranes from

damage caused by salinity. It also improves the plant's ability to regulate water and ion balance and increases the production of antioxidants that protect the plant from oxidative stress [4] Corn (*Zea mays* L.) is a multi-purpose crop that is widely grown in a wide range of climatic zones for food, feed and energy, and plays an important role in the development of the global economy as one of the most consumed cereals. It is classified as a moderately salt-tolerant crop, with a low salt tolerance and is severely affected by salt stress at the seedling stage [5]. The research aims to investigate the effect of biofertilizer and humic fertilizer on the growth and yield of corn irrigated with saline water.

Materials and method

A field experiment was conducted to study the effect of biofertilizer and humic acid on the growth and yield of corn under salt stress conditions. The experiment was carried out in Najaf provainc at the University of Kufa - Agricultural Research Station for the fall season of 2023 AD in a sandy loam soil, classified according to the modern American classification as below the level of the great groups Typic Torrifluent according to the international classification [6.]

Experimental factors:

The experiment included three main factors:

The first factor:

A group of *Klebsiella oxytoca* bacteria that are tolerant to salts was isolated from the zebrafish region of the tarfa plant, which is characterized by its high tolerance to salinity, and it was at two levels (without addition, addition) which represents (B1, B0) [7]. The inoculation was done by mixing the seeds with the inoculum with distilled and sterile water and adding gum arabic after the seeds were washed with distilled and sterile water, then they were sterilized using 1% sodium

hypochlorite solution, after which they were washed again with distilled water to remove traces of the sterilizer and left for half an hour before planting.

Factor 2: Humic fertilizer is added to the soil at levels (0, 4 and 8) kg. H-1, which is symbolized by (H2, H1, H0).

Factor 3: Plants are irrigated with water with salinity levels (1.5, 3 and 6) ds.m^{-1} , which is represented by (S2, S1, S0).

Soil preparation

The soil was plowed using a rotary plough and smoothed using disc harrows. The field was divided into panels with dimensions of 3x2 m for each experimental unit, and a 2.5 m wide

space was left between panels to prevent the transfer of fertilizers and control the movement of water from one treatment to another. Random soil samples were taken from the surface layer 30-0 cm. After removing plant remains, the soil was air dried and ground using a wooden hammer and passed through a sieve with holes diameter of 2 mm and mixed well to homogenize it. Representative samples were taken from it for the purpose of conducting some chemical and physical analyses to study the soil, the results of which are shown in Table (1).

Table (1) Some chemical, physical and biological properties of the soil before planting

units	values	traits	
—	7.59	Reaction degree (pH) 1:1	
DS.m ⁻¹	1.950	Electrical conductivity (EC) 1:1	
g.kg ⁻¹	10.1	Organic matter O.M	
mg.kg ⁻¹	21.00	Nitrogen	Available Elements
	10.10	Phosphorus	
	155	Potassium	
%	9.2	Clay	Prefabricated Elements
	4.0	Silt	
	86.8	sand	
—	Loamy Sand	soil texture	
Mg.m ⁻²	1.43		Bulk density
⁻¹ CFU dry soil	87.00	Total bacterial bioassay	

The seeds of the corn, Euphrates hybrid, which were prepared by the Babylon Agriculture Division / Al-Mahawil, were planted on 7/20/2023 in lines inside plots, with a distance of 75 cm between one line and another and 25 cm between holes and another, at a rate of four lines for each experimental

unit. Between 3-2 seeds were placed in each hole, and the plants were thinned to one plant after two weeks of emergence. The number of plants was 32 plants in the experimental unit, with a plant density of 53.333 plants / ha. The granular diazinon pesticide was used at a concentration of 10% (5 kg / ha) to control the stem borer (*Sesamin Cretica*) twice, the first

time after 20 days of germination and the second time after 15 days of the first control by adding it to the heart of the fourth and third leaves. Nitrogen fertilizers were added at an amount of 250 kg N^{-1} in the form of urea 46% N, phosphate fertilizers at an amount of 100 kg P^{-1} in the form of triple superphosphate fertilizer P 20%, and potassium fertilizers at an amount of 100 kg K^{-1} in the form of potassium sulphate fertilizer K 41%.

Studies traits

-Organic matter:

Estimated by wet digestion method and according to the Walkley and Black method mentioned in [4]

-Available nitrogen:

Available nitrogen was estimated by Microkjeldahl device according to the method mentioned in [4].

-Available phosphorus:

Available soil phosphorus was extracted using sodium bicarbonate (0.5 M (NaHCO_3) at pH = 8.5 according to the Olsen method mentioned in [4]. The color was developed with ammonium molybdate and ascorbic acid, and was estimated using a spectrophotometer at a wavelength of 882 nm.

-Available potassium:

Extracted with ammonium acetate NH_4OAc (1N) and measured by flame photometer according to the method mentioned in [4].

Results and Discussion

Available Nitrogen (mg N kg^{-1} soil)

The results in Table (2) showed that adding biofertilizer gave the highest value with an average of $20.11 \text{ mg. kg}^{-1}$ soil compared to the treatment of biofertilizer without addition, which gave 18.96 mg kg^{-1} soil. It was found that there were no significant differences between the treatment of biofertilizer in the concentration of available nitrogen in the soil

surrounding the roots. It is noted that the addition of humic fertilizer led to a significant increase in the concentration of available nitrogen in the rhizosphere region, as the H2 treatment with an addition level of 8 kg. h^{-1} outperformed both levels 0 and 4 kg. h^{-1} . The concentration of nitrogen in the region near the roots was 17.69, 19.29 and 21.65 mg kg^{-1} soil for each of the levels (0, 4 and 8) kg h^{-1} , respectively, with an increase rate of 22.38 and 9.04% for the level 8 and 4 kg h^{-1} compared to the level 0 kg h^{-1} , respectively. The results of irrigation with water with an electrical conductivity of 6 ds.m^{-1} showed a decrease in the concentration of available nitrogen in the soil surrounding the roots, which reached $8.51 \text{ mg N kg}^{-1}$ soil, compared to the two levels of ds.m^{-1} (1.5 and 3), which reached 27.48 and $22.64 \text{ mg N kg}^{-1}$ soil, respectively, with a conservation rate of 69.03 and 62.41% for each of the two treatments of ds.m^{-1} (1.5 and 3) compared to the irrigation treatment with water with a conductivity of 6 ds.m^{-1} . While significant differences were noted in bi-interaction between biofertilizer and humic fertilizer in the concentration of available nitrogen in the soil, as the highest value reached $22.30 \text{ mg N kg}^{-1}$ soil in treatment H2B1, in contrast, the lowest value reached $17.24 \text{ mg N kg}^{-1}$ soil in treatment H0B0. Also, the interaction between humic fertilizer and salinity levels in irrigation water showed significant differences, the highest value of which reached $29.20 \text{ mg N kg}^{-1}$ represented by treatment H2S0, while the lowest value showed $6.65 \text{ mg N kg}^{-1}$ in treatment H0S2. Also, the triple interaction of all factors showed significant differences, as the highest value reached $29.64 \text{ mg N kg}^{-1}$ represented by treatment H2B1S0, in contrast, the lowest value showed $6.64 \text{ mg N kg}^{-1}$ represented by treatment H0B0S2.

Table (2) The effect of biofertilizer, humic acid fertilizer, irrigation with saline water and their interactions on available nitrogen in the soil (mg N kg⁻¹ soil)

Average interaction of Biofertilizer and Humic	salt water			Humic fertilizer H	Biofertilizer B
	S2	S1	S0		
17.24	6.64	20.10	24.99	H0	B0
18.65	7.04	21.60	27.32	H1	
20.99	10.13	24.10	28.75	H2	
18.14	6.65	21.53	26.23	H0	B1
19.92	8.77	23.06	27.95	H1	
22.30	11.83	25.43	29.64	H2	
3.2661	5.2138			LSD 0.05	
Average Biofertilizer	salt water			Interaction of Biofertilizer and salt water	
	S2	S1	S0		
18.96	7.93	21.93	27.02	B0	
20.11	9.07	23.34	27.94	B1	
				LSD 0.05	
	8.51	22.64	27.48	Average salt water	
2.1958	3.9347			LSD 0.05	
Average humic acid	salt water			Interaction of humic acid and salt water	
	S2	S1	S0		
17.69	6.65	20.82	25.61	H0	
19.29	7.91	22.33	27.64	H1	
21.65	10.98	24.76	29.20	H2	
2.19	2.19			LSD 0.05	

Available phosphorus (mg P kg⁻¹ soil):

The results of Table (3) showed that the biofertilizer treatment was significantly excelled and gave the highest rate of phosphorus in the soil, reaching 26.40 mg P kg⁻¹ soil, while the treatment without adding biofertilizer recorded the lowest rate of available phosphorus in the soil, reaching 22.74 mg P kg⁻¹ soil. It is noted that the addition of humic fertilizer showed significant differences and its average concentration reached 32.41 mg kg⁻¹ soil, achieving the addition level of 8 kg ha⁻¹, while the levels 0 and 4 kg ha⁻¹ showed 18.35 and 22.95 mg P kg⁻¹ soil, respectively, with an increase rate of 76.62% at the H2 level compared to the H0 level and 25.06% for the addition level of 4 kg ha⁻¹ compared to the 0 kg ha⁻¹ level. The results of the studied trait showed a significant decrease in the irrigation treatment with water with a salinity of ds.m⁻¹ 6 by giving the lowest content of available phosphorus, which reached 20.42 mg P kg⁻¹ soil, compared to the treatment of water with an electrical conductivity of ds.m⁻¹ 1.5, which reached 29.91 mg P kg⁻¹, while the treatment of water with an electrical conductivity of ds.m⁻¹ 3 by giving 23.39 mg P kg⁻¹, and the percentage of decrease showed 31.72 and 12.70% for each of S1 and S2 compared to S0. The interaction treatment between biofertilizer and humic fertilizer H2B1 was significantly excelled with the highest content of available phosphorus, which reached 34.27 mg P kg⁻¹ soil, compared to the H0B0 treatment, in which the available phosphorus content decreased, reaching 16.21 mg P kg⁻¹ soil, with an increase rate of 52.69%. Bi-interaction between biofertilizer and irrigation with water showed significant differences, where the treatment of biofertilizer without addition and irrigation with water with electrical conductivity ds.m⁻¹ 6, represented by the symbol S2B0, gave the lowest value, which reached 18.96 mg P kg⁻¹ soil, compared to the treatment of adding biofertilizer and irrigation with water with electrical conductivity ds.m⁻¹ 1.5 S0B1, which gave the highest value, which reached 32.47 mg P kg⁻¹ soil. The interaction between humic fertilizer and irrigation levels was significant and the highest rate of the studied trait was 44.18 mg P kg⁻¹ soil with the addition treatment of 5 kg H-1 and the irrigation treatment with water with an electrical conductivity of ds.m⁻¹ 1.5, symbolized by H2S0, while the lowest rate of the studied trait content was 15.29 mg P kg⁻¹ with the humic fertilizer level of 0 kg H-1 and the irrigation treatment with water with an electrical conductivity of ds.m⁻¹ 6. The results of Table (3) of the interaction between the three factors indicate the presence of significant differences, and the addition of biofertilizer B1 and humic fertilizer at a level of 8 kg H-1 H2 and irrigation with water at a degree of ds.m⁻¹ 1.5 S0 gave the highest rate of available phosphorus, which was 48.43 mg P kg⁻¹ Soil, while the biofertilizer without adding B0 and humic fertilizer at the level of adding 0 kg ha⁻¹ and irrigation with electrical conductivity ds.m⁻¹ 6 gave a percentage of 13.05 mg P kg⁻¹ soil.

Table (3) Effect of biofertilizer, humic acid fertilizer, irrigation with saline water and their interactions on available phosphorus in the soil (mg P kg⁻¹ soil)

Average interaction of Biofertilizer and Humic	salt water			Humic fertilizer H	Biofertilizer B
	S2	S1	S0		
16.21	13.05	17.60	17.97	H0	B0
21.46	19.90	20.35	24.12	H1	
30.56	23.94	27.81	39.93	H2	
20.50	17.53	20.17	23.81	H0	B1
24.45	23.31	24.86	25.18	H1	
34.27	24.83	29.54	48.43	H2	
4.5314	0.797			LSD 0.05	
Average Biofertilizer	salt water			Interaction of Biofertilizer and salt water	
	S2	S1	S0		
22.74	18.96	21.92	27.34	B0	
26.40	21.89	24.86	32.47	B1	
0.2763	5.4147			LSD 0.05	
	20.42	23.39	29.91	Average salt water	
	7.81			LSD 0.05	
Average humic acid	salt water			Interaction of humic acid and salt water	
	S2	S1	S0		
18.35	15.29	18.88	20.89	H0	
22.95	21.60	22.59	24.65	H1	
32.41	24.39	28.68	44.18	H2	
0.33	0.33			LSD 0.05	

available potassium (mg K kg⁻¹ soil:(

The results in Table (4) showed significant differences in the concentration of potassium in the rhizosphere soil in the biofertilizer treatment, as the treatment of adding biofertilizer B1 was significantly excelled and gave the highest average of 182.22 mg K kg⁻¹ soil, while control treatment (without adding biofertilizer) gave the lowest average of 134.72 mg K kg⁻¹ soil, with an increase rate of 35.25% for the addition treatment compared to the non-addition treatment.

The results also showed that adding humic fertilizer had a significant effect on the available potassium, as the addition level exceeded 8 kg h⁻¹ and gave the highest rate of available potassium, reaching 206.67 mg K kg⁻¹ soil, compared to the treatment without adding H0, which recorded the lowest rate of available potassium, reaching 105.82 mg K kg⁻¹ soil. The results showed that the use of saline water led to a significant decrease in the available potassium in the soil, as the values reached (203.61, 153.09 and 118.71) mg K kg⁻¹ soil for each of the different salt concentrations at levels (1.5, 3 and 6) dS.m⁻¹, respectively, with a decrease rate of 41.69% for the treatment of water with an electrical conductivity degree of 1.5 ds.m⁻¹. Table (4) indicated that there were significant differences in the interaction between biofertilizer and humic fertilizer in the availability of potassium in the rhizosphere region, as the treatment of adding biofertilizer + adding humic fertilizer at a level of 8 kg ha⁻¹ gave the highest rate of 237.22 mg K kg⁻¹ soil, while control treatment of biofertilizer without addition and humic fertilizer treatment at a level of 0 kg ha⁻¹ gave the lowest rate of

90.70 mg K kg⁻¹ soil, with an increase rate of 161.53%. The interaction between biofertilizer and irrigation with water with different levels of salinity showed significantly excelled, as the treatment of adding biofertilizer, which is B1, and the irrigation treatment with water with an electrical conductivity of ds.m⁻¹ 1.5 gave the highest average of 233.05 mgK kg⁻¹ soil, while the treatment of biofertilizer without addition and the irrigation treatment with saline water at the level of ds.m⁻¹ 6 showed an average of 104.57 mgK kg⁻¹ soil. The dual interaction between humic fertilizer and irrigation with saline water showed significant differences in potassium availability in the rhizosphere, as the treatment of humic fertilizer at a level of 8 kg ha⁻¹ + irrigation with water at an electrical conductivity of ds.m⁻¹ 1.5 gave the highest average of 247.84 mgK kg⁻¹ soil, and control treatment of humic fertilizer at a level of 0 kg H-1 and irrigation with water with an electrical conductivity of ds.m⁻¹ 6 had the lowest average, reaching 75.18 mgK kg⁻¹ soil. It is noted that the triple interaction between biofertilizer, humic fertilizer and irrigation with saline water showed significant differences, as the treatment of adding biofertilizer and humic fertilizer at an addition level of 8 kg H-1 and irrigation with water with an electrical conductivity of ds.m⁻¹ 1.5 gave the highest average of 289.19 mgK kg⁻¹ soil, in contrast, the treatment of biofertilizer without addition and humic fertilizer at an addition level of 0 kg H-1 and irrigation with water with an electrical conductivity of ds.m⁻¹ 6 showed the lowest average, reaching 69.26 mgK kg⁻¹ soil.

Table (4) The effect of biofertilizer, humic acid fertilizer, irrigation with saline water, and their interactions on available potassium in the soil (mg K kg/m³ soil)

Average interaction of Biofertilizer and Humic	salt water			Humic fertilizer	Biofertilizer
	S2	S1	S0		
90.70	69.26	76.79	126.06	H0	B
137.33	88.15	133.90	189.93	H1	
176.11	156.30	165.55	206.48	H2	
120.93	81.09	94.84	186.87	H0	B0
188.52	136.89	205.58	223.10	H1	
237.22	180.58	241.89	289.19	H2	
0.3046	0.5917			LSD 0.05	
Average Biofertilizer	salt water			Interaction of Biofertilizer and salt water	
	S2	S1	S0		
134.72	104.57	125.41	174.16	B0	
182.22	132.85	180.77	233.05	B1	
0.1328	0.4056			LSD 0.05	
	118.71	153.09	203.61	Average salt water	
	0.3716			LSD 0.05	
Average humic acid	salt water			Interaction of humic acid and salt water	
	S2	S1	S0		
105.82	75.18	85.81	156.47	H0	
162.93	112.52	169.74	206.52	H1	
206.67	168.44	203.72	247.84	H2	
0.2575	0.5203			LSD 0.05	

Organic matter in the soil (g/kg/soil: (

The data in Table (5) showed that there are significant differences in the percentage of organic matter in the study soil surrounding the root. The treatment of adding biofertilizer, which is represented by the symbol B1, gave the highest rate of organic matter in the soil, amounting to 11.06 g/kg-soil, compared to the treatment of biofertilizer without addition, which gave the lowest average, amounting to 9.57 g/kg-soil, which is represented by the symbol B0, and with a significant increase rate of 15.88% for the treatment of adding biofertilizer compared to not adding.

As for the addition of humic fertilizer and its effect, it was found that the treatment of humic fertilizer at a level of 8 kg.ha⁻¹ was significantly excelled with a value of 12.18 g kg⁻¹ soil compared to levels 0 and 4 kg ha⁻¹, which gave 8.40 and 10.37 g kg⁻¹ soil, respectively, and with an increase rate of 45.00 and 23.45% for the addition level of 8 and 4 kg ha⁻¹ compared to the addition level of 0 kg ha⁻¹, respectively. The results showed that the use of water at different levels of electrical conductivity significantly decreased the values of organic matter in the soil, as it reached (12.38, 10.10 and 8.47) g kg⁻¹ soil for each of the salinity levels (S2, S1, S0) respectively. The decrease rate reached 31.58 and 3% 16.1 for both S2 and S1 compared to S0. It is noted that the results of bi-interaction between biofertilizer and humic fertilizer showed significant differences. The treatment of adding biofertilizer with humic fertilizer at a level of 8 kg ha⁻¹ gave the highest average of organic matter, reaching 13.08 g. kg⁻¹ soil, while the lowest average was 7.63 in the

treatment of biofertilizer without addition and humic fertilizer at an addition level of 0 kg ha⁻¹. It was noted that bi-interaction between biofertilizer and irrigation with saline water showed a significant effect on the values of organic matter, as it gave the highest average in the treatment of biofertilizer at the addition level and the treatment of saline water at a level of ds.m⁻¹ 1.5, reaching 12.89 g. kg⁻¹ soil for organic matter, in contrast, control treatment showed the lowest rate of 7.71 g. kg⁻¹ soil for the biofertilizer without addition and the irrigation treatment with saline water at a level of ds.m⁻¹ 6. While it is noted that the results of bi-interaction between humic fertilizer and irrigation with water showed significant differences, the treatment of adding biofertilizer in combination with irrigation with water with an electrical conductivity of ds.m⁻¹ 1.5 gave the highest value with an average of 14.80 g. kg⁻¹ soil compared to the treatment of biofertilizer without addition and irrigation with a salinity level of ds.m⁻¹ 6, which gave the lowest value with an average of 6.21 g. kg⁻¹ soil. The results of the triple interaction between the three factors also indicated that the treatment of adding biofertilizer + humic acid fertilizer at a rate of 8 kg ha⁻¹ + saline water ds.m⁻¹ 1.5, which is symbolized by (H2B1S0), achieved the highest readiness of organic matter in the soil, reaching 15.73 g kg⁻¹ soil, compared to the treatment of biofertilizer without addition + humic acid fertilizer at a rate of 0 kg ha⁻¹ + saline water ds.m⁻¹ 6, which is symbolized by (H0B0S2), which gave the lowest readiness of organic matter, reaching 5.13 g kg⁻¹ soil.

Table (5) The effect of biofertilizer, humic acid fertilizer, irrigation with saline water and their interactions on organic matter in the soil (g kg⁻¹ soil)

Average interaction of Biofertilizer and Humic	salt water			Humic fertilizer H	Biofertilizer B
	S2	S1	S0		
7.63	5.13	7.73	10.03	H0	B0
9.82	8.33	9.40	11.73	H1	
11.28	9.66	10.33	13.86	H2	
9.17	7.30	9.73	10.50	H0	B1
10.93	9.30	11.03	12.46	H1	
13.08	11.13	12.40	15.73	H2	
1.1388	0.8325			LSD 0.05	
Average Biofertilizer	salt water			Interaction of Biofertilizer and salt water	
	S2	S1	S0		
9.57	7.71	9.15	11.87	B0	
11.06	9.24	11.05	12.89	B1	
	8.47	10.10	12.38	Average salt water	
0.2955	1.7902			LSD 0.05	
Average humic acid	salt water			Interaction of humic acid and salt water	
	S2	S1	S0		
8.40	6.21	8.73	10.26	H0	
10.37	8.81	10.21	12.10	H1	
12.18	10.40	11.36	14.80	H2	
0.3619	2.0376			LSD 0.05	

Total number of bacteria in the rhizosphere soil (gm^{-1} dry soil) of 6.68 and 4.30 gm^{-1} dry soil respectively, with a decrease rate of 47.56 and 35.63% for the water level ds.m^{-1} 1.5 and 3 compared to the level 6 ds.m^{-1} . Table (6) indicated that bi-interaction between biofertilizer and humic fertilizer gave the highest value for bacterial numbers in the rhizosphere region, reaching 8.02 gm^{-1} dry soil when adding biofertilizer and humic fertilizer at a level of 8 kg ha^{-1} , in contrast, it gave the lowest value, reaching 5.44 gm^{-1} dry soil in the treatment of biofertilizer without adding and humic fertilizer at a level of 0 kg ha^{-1} . It is noted that bi-interaction between humic fertilizer and irrigation with water showed the highest value, reaching 8.93 gm^{-1} dry soil when treating humic fertilizer at a level of 8 kg ha^{-1} and with an electrical conductivity of ds.m^{-1} 1.5, in contrast, it showed the lowest value, reaching an average of 3.59 gm^{-1} dry soil when treating humic fertilizer at a level of 0 kg ha^{-1} and irrigation water at an electrical conductivity of ds.m^{-1} 6. Table (6) also indicated that The triple interaction between biofertilizer, humic acid fertilizer and irrigation with saline water reached the highest average of 9.33 gm^{-1} dry soil in the H2B1S0 treatment compared to the H0B0S2 treatment, which reached an average of 3.36 gm^{-1} dry soil.

The results in Table (6) showed that biofertilization led to a significant increase in the number of bacteria in the rhizosphere region if the treatment with addition was superior at an average rate of 6.67 gm^{-1} soil compared to the treatment with biofertilizer without addition, which gave the lowest rate of 6.12 gm^{-1} soil and an increase rate of 8.25% for the addition treatment compared to the non-addition treatment.

It is noted from the above-mentioned characteristic that humic fertilizer had a significant effect in increasing the number of bacteria in the area surrounding the roots and gave the highest value for the number of bacteria in the treatment with an addition level of 8 kg.ha^{-1} , as its average reached 7.50 gm^{-1} dry soil compared to the addition level (0 and 4) kg.ha^{-1} , which gave an average of 5.54 and 6.13 gm^{-1} dry soil, respectively, with an increase rate of 35.38% for the level of 8 kg.ha^{-1} compared to the level of 0 kg.ha^{-1} and 10.65% for the level of 4 kg.ha^{-1} compared to the level of 0 kg.ha^{-1} . It is noted from Table (6) that there is a significant decrease in the water treatment, as the water treatment at the level of ds.m^{-1} 1.5 showed the highest value with an average of 8.20 gm^{-1} dry soil compared to the water treatment at levels (3 and 6) ds.m^{-1} which gave the lowest average

Table (6) Effect of biofertilizer, humic acid fertilizer, irrigation with saline water and their interactions on the number of bacteria in the soil (CFU $\times 10^4$ gm⁻¹ dry soil)

Average interaction of Biofertilizer and Humic	salt water			Humic fertilizer	Biofertilizer B
	S2	S1	S0		
	H				
5.44	3.36	5.38	7.60	H0	B0
5.93	3.93	6.20	7.68	H1	
6.99	4.70	7.76	8.53	H2	
5.64	4.83	5.63	7.46	H0	B1
6.34	4.10	6.33	8.60	H1	
8.02	5.91	8.83	9.33	H2	
0.6103	0.0939			LSD 0.05	
Average Biofertilizer	salt water			Interaction of Biofertilizer and salt water	
	S2	S1	S0		
6.12	3.99	6.44	7.93	B0	
6.78	4.97	6.93	8.46	B1	
				LSD 0.05	
	4.47	6.68	8.20	Average salt water	
0.0316	1.7902			LSD 0.05	
Average humic acid	salt water			Interaction of humic acid and salt water	
	S2	S1	S0		
5.54	3.59	5.49	7.53	H0	
6.13	4.01	6.26	8.14	H1	
7.50	5.30	8.29	8.93	H2	
0.3619	1.0162			LSD 0.05	

The results in Table (3-6) showed that adding biofertilizer had a significant effect on increasing the content of available nitrogen, phosphorus, potassium, organic matter, and bacterial numbers in the rhizosphere compared to their content before planting. This is attributed to the fact that biofertilizer settles in the rhizosphere, which is the area surrounding the roots and where most of the biological activities take place. Due to its biological activity, plant growth improves by raising the availability of some important elements for the plant [7]. Biofertilizer may have a limited effect on soil fertility and nitrogen availability. This is consistent with what [11] found that direct inoculation of bacteria in the soil may face difficulty in colonizing and surviving around the roots of yellow corn because it is exposed to a variety of environmental factors, such as competition from native microflora, unfavorable physical and chemical conditions, and fluctuations in pH and temperature, since nitrogen is a mobile element in the soil, especially since irrigation is one of the factors that affect its survival for a longer period. There are many factors that work to wash nitrogen in the soil, in addition to different types of stresses. [12] stated that biofertilizers settle in the rhizosphere, which is the area surrounding the roots and where most of the biological activities take place. Due to their biological activity, plant growth improves by increasing the availability of some important elements for the plant. [7] also stated that the movement of phosphorus in the soil solution is very slow and phosphate is often precipitated with positive ions. These precipitation processes can be controlled by adding organisms that secrete enzymes that increase the availability of phosphorus in the soil continuously throughout its life. Biofertilizers are characterized by their role in secreting

organic and inorganic acids that work to reduce the values of the degree of interaction, which in turn increases the forms of phosphorus that are most available to the plant and increases the readiness of nutrients in the soil, as biofertilizer works to improve the properties of the soil and then balance the nutrients in the root zone and convert them into the ready form. It is noted that adding *Klebsiella oxytoca* bacteria mixed with seeds plays a role in converting the phosphorus available in the soil into forms that are more absorbable by the plant through the secretion of the phosphatase enzyme, which works to dissolve phosphorus from its fixing sources and thus increases its readiness in the soil. These results are consistent with what was reached by [15], and potassium also increases plant growth, including increasing the root system, which is reflected in increasing its respiration and increasing the secretion of CO_2 , which combines with water to form carbonic acid, which begins to dissolve the minerals carrying phosphorus, increasing their release, which increases their readiness. Phosphorus in soil [16.]

These organisms play an important role in sustainable development through the exchange between types of biofertilizers, organic and chemical and their impact on plant production and quality [17]. These bacteria increase microorganisms and inhibit microorganisms harmful to plants and soil, which causes growth and reproduction. Root secretions are a source of food for *Klebsiella oxytoca* bacteria that live and reproduce in this environment. In addition to the fact that these organisms were isolated locally from saline soil and may have had some familiarity and adaptation to the conditions of agricultural soil. All of the above can be attributed to the reason for the increase in the content of available nitrogen,

phosphorus, potassium, organic matter and the number of bacteria in the rhizosphere region, The addition of humic fertilizer achieved a significantly excelled in the above-mentioned characteristics, due to its important role in supplying the soil with an initial concentration of nutrients, in addition to the role of these acids in the process of converting formulas of nutrients present in the soil into formulas available for absorption by the plant. Humic fertilizer can also improve the effect of urea fertilization by stabilizing urease [18]. The addition of organic fertilizers had a clear effect in increasing the percentage of nutrients in the leaves, and this increase may be due to the fact that these extracts contain major and minor nutrients that are absorbed either directly through the leaves or through the roots, Tables (2 and 3), and then increase their percentage in the plant. The added humic acids contain nutrients including nitrogen, and are considered a rich source of phosphorus and humic acids increase the plant's ability to absorb nutrients such as nitrogen, phosphorus, potassium, iron, magnesium, copper, zinc, etc., which results in increased growth of the plant's vegetative and root system [19]. It is concluded from the tables that the mineral fertilizers added to both experiments had a significant effect on the availability of nutrients (nitrogen, phosphorus, potassium, magnesium, and calcium) and their absorption by seedlings and increased their percentage in the leaves, which is consistent with [9]. The addition of humic acid led to the release of organic nitrogen in the soil by increasing microbial activity and enzymes that decompose proteins and nucleic acids. It was noted that the increased availability of major nutrients in humic fertilizer is slow-release and has unique chemical and physical properties and is more capable of meeting root

demand efficiently through the ion exchange process. Humic fertilizer also works to preserve the availability of nutrients and reduce external influences associated with the plant's need. The addition of humic acid led to the release of CO_2 and its dissolution in water formed carbonic acid, which led to a reduction in the degree of soil reaction in addition to the formation of phosphorus compounds that prevent phosphorus precipitation and work to increase the dissolution of calcium phosphate and the release of ready phosphorus, thus increasing the soil content of ready phosphorus in the presence of humic fertilizer. The addition of nitrogenous fertilizers increases the availability of phosphorus in the soil and thus increases its absorption by the plant [22] Increasing the surface area in humic fertilizer plays a role in increasing soil aeration [20]. Humic acid fertilizer produces organic acids capable of dissolving minerals and potassium-bearing compounds, thus increasing the availability of potassium. These results are consistent with what was reached by [20]

Salt levels affected the experimental coefficients for the studied traits, as it led to a decrease in the availability of nutrients and the availability of ions that bind to phosphate such as calcium ions, and sodium ions instead of potassium ions, which leads to its precipitation and then conversion into forms that are not ready for absorption by the plant, as [21] concluded that the increase in the salinity of irrigation water, the more it increases, the less the amount of available nitrogen in the soil, as well as [4] who found that when the concentration of salts increased, it led to a decrease in the increase in the availability of elements in the soil. It led to a reduction in the activity of organisms and thus reduced the rate of decomposition of organic matter, especially

the effect of sodium and chloride ions, which leads to a reduction in microbial growth in saline soil. These results are consistent with what was reached by [22] who found that the

References

.1 Hassan, M. U., Kareem, H. A., Hussain, S., Guo, Z., Niu, J., Roy, M., ... & Wang, Q. (2023). Enhanced salinity tolerance in Alfalfa through foliar nano-zinc oxide application: Mechanistic insights and potential agricultural applications. *Rhizosphere*, 28, 100792.

.2 Khan, A. A., Khan, S. U., Ali, M. A. S., Khan, A., Hayat, Y., & Luo, J. (2022). Drivers of climate variability and increasing water salinity impacts on the farmer's income risk with future outlook mitigation. *International Journal of Climate Change Strategies and Management*, 14(5), 462-485.

.3 Zhang, H., Zhang, Y., Wang, Y., Liu, X., & Liu, Z. (2021). "The role of biofertilizers in sustainable agriculture: A review." **Agronomy for Sustainable Development**, 41(2), 25. DOI: [10.1007/s13593-021-00636-2](https://doi.org/10.1007/s13593-021-00636-2) (

.4 Sadiq, A., & Ahmad, R. (2021). "The Role of Humic Acid in Mitigating Salinity Stress in Plants: A Review." **Journal of Plant Nutrition**, 44(10), 1457-1475. doi:10.1080/01904167.2021.1874589.

.5 Rostami.M.; A .Shokouhian and Mohebodini.M.,2022. Effect of Humic Acid, Nitrogen Concentrations and Application Method on the Morphological, Yield and Biochemical Characteristics of Strawberry 'Paros'. Volume 22. 203-214.

.6 Sivasakthivelan, P. and P. Saranraj.2013. *Azospirillum* and its

increase in the salinity of irrigation water works to inhibit the decomposition of organic matter, and thus its percentage decreases with the rise in saline water levels.

Formulations: A Review. *Intl. J. Microbiol. Res.*, 4 (3): 275-287. Soil Survey Staff. Key to soil taxonomy. 10th edition.

.7 Khalil, S.E., Abdel-Aziz, N.H., El-Nagar, M.E. (2018). Effect of *Klebsiella oxytoca* on nutrient uptake and yield of maize plants. *Egyptian Agricultural Journal*, 96(1), 85-95

.8 Mackowiak, C. L., Grossl, P. R., & Bugbee, B. G. (1999). Beneficial effects of humic acid on micronutrient availability to wheat. *Soil Science Society of America Journal*, 63(6), 1665-1671.

.9 Page, A.L., Miller, R.H. and Keeney, D.R. (1982) *Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties*. American Society of Agronomy. In *Soil Science Society of America*, Vol. 1159.

.10 Sylvia, D. M., Fuhrmann, J. J., Hartel, P. G., & Zuberer, D. A. (2005). *Principles and applications of soil microbiology* (pp. 373-404). Pearson.

.11 Parida S.K and A.B. Das . 2005. Salt tolerance and salinity effects on plants, *Ecotoxicol. Environ. Safety*. 60 (3) : 324-349 .

.12 Bhat, M.A.; R. Rasool and Ramzan.S. 2019. Plant Growth Promoting Rhizobacteria (PGPR) for Sustainable and Eco-Friendly Agriculture. *Acta Scientific Agriculture* 3(1): 23-25 .

.13 Subramanian, K. S., & Thirunavukkarasu, M. (2017). Nano-fertilizers and nutrient transformations in soil. *Nanoscience and plant-soil systems*, 305-319.

.14 Khan, M. I., & Qureshi, M. I. (2022). "Salt Stress in Plants: Mechanisms and

Management Strategies." **Agronomy**, 12(5), 1150.

<https://doi.org/10.3390/agronomy12051150>

.15 Gallo, A., Zannoni, D., Valotto, G., Nadimi-Goki, M., & Bini, C. (2018). Concentrations of potentially toxic elements and soil environmental quality evaluation of a typical Prosecco vineyard of the Veneto region (NE Italy). *Journal of Soils and Sediments*, 18, 3280-3289.

.16 Al-Mashhadani, Walid Ali Hamid. 2021. The effect of some nano-nutrient fertilizers, humic acids and biofertilizers on the availability of some major and minor elements and the growth and yield of wheat (*Triticum aestivum* L.). College of Agricultural Engineering Sciences, University of Baghdad. Republic of Iraq.

.17 Mohsen, N., Khalil, M. N., Moussa, K. F., & El-Naka, E. A. (2017). INTERACTION EFFECT OF SOME SOIL AMENDMENTS ON THE QUALITY OF SANDY SOILS CULTIVATED WITH WHEAT. *Zagazig Journal of Agricultural Research*, 44(4), 1339-1357.

.18 Laskosky, J. D., Mante, A. A., Zvomuya, F., Amarakoon, I., & Leskiw, L. (2020). A bioassay of long-term stockpiled

salvaged soil amended with biochar, peat, and humalite. *Agrosystems, Geosciences & Environment*, 3(1), e20068.

.19 Khaled, H., & Hassan F, H. A. (2011). Effect of different levels of humic acids on the nutrient content, plant growth, and soil properties under conditions of salinity. *Soil and Water Research*, 6(1), 21.

.20 Jweer. K. A.; I. A. Abed.; H. Bardan and Hamad.A.2021. Effect of Local Lypreparedorganic Fertilizers by Support with Phosphate Rock and Decomposed Biology in the Production of Seedlings and the Yield of the Lettuce Plant.25.16.Lehmann, Johannes .2007a. "Bio-energy in the black". *Front Ecol Environ* 5 (7.(

.21 Rietz DN and Haynes RJ.2003. Effects of irrigation-induced salinity and sodicity on soil microbial activity. *Soil Biol Biochem*. 2003;35(6):845-854. DOI: 10.1016/s0038-0717(03)00125-1.

.22 Walpola . B. C. and Arunakumara .K. K. I. U. 2010. Effect of salt stress on decomposition of organic matter and nitrogen mineralization in animal manure amended soils. *The Journal of Agricultural Sciences*. 5 (1): 9-18.